PB80-209505

Failure to Produce Arsenic Neurotoxicity in the Rat An Experimental Study

American Public Health Association
Washington, DC

Prepared for

Environmental Protection Agency
Washington, DC

May 80

U.S. Department of Commerce
National Technical Information Service
NTIS.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECI... PB80-209505
4. TITLE AND SUBTITLE Failure to produce arsenic neurptoxicity in the rat.An Experimental Study		5. REPO... May 1980 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Herbert H. Schaumburg		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS American Public Health Association 1015 Fifteenth Street, N.W. Washington, D.C. 20005		10. PROGRAM ELEMENT NO. 11. CONTRACT/GRANT NO. EPA 560/11-80-022
12. SPONSORING AGENCY NAME AND ADDRESS Office of Toxic Substances U.S. Environmental Protection Agency 401 M St., S.W. Washington, D.C. 20460		13. TYPE OF REPORT AND PERIOD COVERED Final Report 14. SPONSORING AGENCY CODE

15. SUPPLEMENTARY NOTES

16. ABSTRACT

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It appears that the rat is not the appropriate species for the study of inorganic arsenic neurotoxicity.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Laboratory Study (18 Months) Neurotoxicity in the Rat Inorganic Arsenic Histopathological	Arsenic Trioxide Intraperitoneal Injections (2mg/kg,5mg/kg, 10mg/kg, 15mg/kg	

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
UNLIMITED	Unclassified	
	20. SECURITY CLASS *(This page)*	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

PB80-209505

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

EPA 560/11-80-022
May 1980

FAILURE TO PRODUCE ARSENIC NEUROTOXICITY IN THE RAT AN EXPERIMENTAL STUDY

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DISCLAIMER

This project has been funded with Federal funds from the Environmental Protection Agecny under contract number 68-01-3859. The content of this publication does not necessarily reflect the views or policies of the U.S. Environmental Protection Agency, nor does memtion of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

ABSTRACT

Prolonged, low-level exposure to inorganic arsenic can produce peripheral neuropathy in humans, and is a serious industrial health hazard. The clinical expression of arsenic neuropathy is similar to other toxic neuropathies of the dying-back type. No satisfactory animal model of arsenic neuropathy has been devised.

Rats underwent weekly intraperitoneal injections with solutions of arsenic trioxide. Strengths of 2mg/kg, 5mg/kg, 10mg/kg and 15mg/kg were used. The 15mg/kg animals died shortly after receiving the injection. The other animals survived and, after eighteen months, appeared normal. Histopathological study of the peripheral and central nervous systems of these animals was unremarkable.

It appears that the rat is not the appropriate species for the study of inorganic arsenic neurotoxicity.

I. HISTORICAL BACKGROUND

Inorganic arsenic was utilized as a form of therapy by the ancient Greek and Roman physicians. Epidemics of polyneuritis in the past suggest the possibility of widespread arsenical poisoning. An epidemic of 40,000 cases of arsenism occurred in France in 1828 when arsenious acid (arsenic trioxide dissolved in dilute hydrochloric acid) was accidentally introduced into wine and bread. In England in 1900, there was an epidemic of arsenic neuropathy in which an estimated 6000 cases and approximately 70 deaths occurred. This outbreak or arsenism resulted from the use of arsenic-contaminated sugar in the preparation of beer. A comparable outbreak followed the use of malt that was contaminated with arsenic as the consequence of having been dried with coke gas. Arsenic trioxide is a product of smelting operations, and it is the starting material for synthesizing most arsenical compounds. In the natural environment, most arsenicals degrade or weather to form arsenate.

Tolerance to arsenic varies, and this has been attributed to individual idiosyncrasies. Levels of 0.05 to 0.30 ppm are normally found in most tissues, similar to levels found in the rabbit. Arsenic eating habitually took place in Styria and Tyrol. Persons who ate arsenic could tolerate dosages of approximately 5 mg per day; more habitual arsenic eaters could tolerate 500 mg in a single oral dose. The medicinal use of Fowler's solution was more common in the past, and excessive administration of such arsenical medications led to a neuropathy. In addition to accidental poisoning as a result of contamination of bread, wine, or beer, arsenical intoxication has been due to occupational exposure in a number of industries --- for example, power production, leather manufacturing, paint and dye production, and the preparation of insecticides.

Biological and Clinical:

Arsenic neuropathy is the most common type of metal neuropathy and, by its clinical expression, is clearly of the dying-back type. Many times, the source of the arsenic remains a mystery. With a mild neuropathy, the patient may mention only the presence of mild paresthesias, usually of the feet. With severe involvement of the peripheral nerves, there is a severe sensory of sensorimotor polyneuropathy with a stocking-glove pattern of hyperesthesia or anesthesia and distal weakness, both of which may preclude ambulation. Often the patient will complain of intense pain or painful paresthesiase, tenderness, and swelling of the hands and feet, more troublesome in the lower limbs than in the upper. Mild neuropathies may be painless. When the upper limbs are involved, the medial and ulnar nerves are more affected than the other upper limb nerves. Paralysis of the facial nerve and motor portion of the trigeminal nerve has also been reported. At times, with chronic poisoning, there is a brownish discoloration of the skin that, on the trunk, may assume a teardrop appearance with a dermatome distribution. The skin lesions have been described a herpetiforn or pemphigoid. The soles, and at times the hands, become darkened and hyperkeratotic, and these changes can be followed by skin cancer. Another variation is that the hands or feet become swollen, blotchy, reddened or livid, and hyperhidrotic. The nails are sometimes brittle and thin, with the appearance of transverse whitish striae (Mees' lines). Mees' lines appear approximately a month after the ingestion of arsenic.

Chronic arsenical intoxication, either accidental or intentional, is insidious in onset and is characterized by nonspecific symptoms such as malaise, nausea, anorexia, generalized weakness, intermittent diarrhea, and constipation. Upper respiratory symptoms also can be present, as well as increased salivation and stomatitis. Keratosis of the palms and soles with increased pigmentation (arsenical melanosis) is characteristic of chronic exposure to arsenic. Heptomegaly, jaundice, and changes in the liver compatible with cirrhosis can be found. The toxic effect on the bone marrow leads to anemia, usually of the aplastic type.

2. EXPERIMENTAL NEUROPATHOLOGY:

The structural changes of the peripheral nervous system caused by experimental arsenic intoxication have not been studied very thoroughly. In a study of spinal cords of dogs after arsenic intoxication performed in 1883[1], vacuoles and other abnormalities appeared in anterior horn cell neurons. These probably represent fixation artifact. Except for one study in pigs[2], in which no histology was illustrated, no satisfactory animal model has been devised.

3. HUMAN NEUROPATHOLOGY:

Erlicki and Rybalkin's study of two human cases in 1892 probably represents the most extensive and reliable evaluation of the morphologic changes in arsenic neuropathy[3]. The cases were carefully studied clinically over many months, and extensive pathologic studies were done in one case. In both cases, the time of the accidental ingestion of arsenic was known. In addition to the initial abdominal pain, vomiting, and subsequent delirium, pain described as burning, searing, tearing, stabbing, and cutting and of extreme severity developed in about one week. The pain was associated with decreased touch-pressure, temperature discrimination, and pain sensations, and was located in the soles of the feet, toes, lowerlimbs, hands, and fingers. It was much accentuated by putting weight on the feet or scratching the sole. Muscle weakness began 3 to 4 days after arsenic ingestion and reached its greatest severity at approximately 12 days. The greatest weakness was in the distal muscles of the lower limb, more in small foot muscles and peroneal muscles than in anterior tibial, calf, or thigh muscles. In the more severe case, more proximal muscles were affected also. In one case, recovery of muscle strength began at six weeks in proximal muscles. In the milder case, by one and a half years muscle strength had returned except for the extensor muscles of the ulnar three fingers; in lower limbs also, improvement had occurred so that walking

was possible with a cane. The more severely affected patient died from pneumonia during the prolonged convalescence. Although incontinence occurred during the period of delirium, bowel and bladder function were not affected. Tendon reflexes, particularly of the lower limbs, were absent. In the milder case the knee reflex returned a year and a half after the exposure.

At autopsy in the more severe case, pathologic changes were found in the spinal cord and peripheral nerves. Anterior horn cells were decreased in number in particular groups of the ventral gray matter and also diffusely. None of the remaining cells was normal: they had lost their angular contours, were rounded, and often lacked processes; the cytoplasm was altered; and the normal "stripes" had degenerated into granular yellow or yellow-brown pigment. This last change may reflect nothing more than the presence of lipofuscin, an abnormality occurring in spinal cords unaffected by specific diseases. Nuclei were ragged and sometimes pale. In the cervical and lumbar enlargements of the spinal cord there was no marked abnormality except that posterior and anterior columns were markedly thinned and had decreased numbers of myelinated fibers. Only the radial and peroneal nerves were evaluated. The majority of myelinated fibers were abnormal, but the morphologic changes were not described in detail. In the authors' opinion, some fibers had remained without degeneraton, some were degenerating, and others were regenerating. Unequivocal evidence of both central and peripheral pathologic changes was thought to be present.

Longo and co-workers[4] reported extensive degeneration in distal ramifications of peripheral nerve fibers, as seen in wallerian degeneration. In a series of 708 patients with neuropathy, Chhuttani and co-workers (1967)[5] found 40 in whom the neuropathy could be blamed on arsenic. Biopsy of a digital nerve of the toe showed increased cellularity, increased thickness of perineurium, and a decreased number of and degeneration of myelinated fibers.

4. PURPOSE OF THIS STUDY AND JUSTIFICATION:

This study was designed to produce chronic inorganic arsenic intoxication in rats, resulting in a peripheral neuropathy resembling the human neuropathy associated with prolonged low level environmental or industrial exposure. Our purpose was to study the spatial temporal evolution of peripheral and central nervous system disease. No satisfactory animal model of human arsenic intoxication exists (see background).

Several benefits would stem from a morphological study of an experimental animal model:

1) The distribution of the neuropathological changes would indicate the vulnerable areas of the nervous system, and greatly facilitate clinical evaluation of humans exposed to arsenic. This would be especially useful in suggesting the proper clinical test used in a screening operation amongst workers exposed to arsenic.

2) The character of the neuropathological changes might suggest the nature of the biochemical abnormality underlying arsenic neurotoxicity, and facilitate prevention or treatment of arsenic neuropathy.

5. METHODS:

Eighteen young male adult Sprague-Dawley rats were used in this study. Fifteen animals underwent weekly intraperitoneal injections with solutions of arsenic trioxide. These 15 animals were divided into 4 groups depending on the strength of the solution injected 15 mg/kg, 10 mg/kg, 5 mg/kg, and 2 mg/kg. Three animals were used as controls, and received weekly injections of normal saline. All animals were allowed food and water ad libitum, examined daily and weighed weekly.

Perfusion and Tissue Preparation:

At the completion of the period of intoxication, each animal was anesthetized with sodium barbitone containing heparin, the chest opened and the animal perfused with fixative (phosphate-buffered 4% paraformaladehyde for 1 minute at 100mm/Hg pressure followed by phosphate-buffered 5% glutaraldehyde at 100-180 mm/Hg for 15 minutes) by a cannula, placed into the apex of the heart, opening into the aortic arch, and draining via the opened right atrium. Tissue was sampled (vide infra) and immersed in Dalton's chrome osmium tetroxide for one hour of postfixation. After alcohol dehydration, epon infiltration, block hardening and trimming, one micrometer sections were cut with a glass knife in a Porter Blum MT2B, mounted on glass slides and stained with a 1% aqueous solution of toluidine blue with 0.5% borax for 1 minute at 300°C. These slides were examined with a light microscope. To prepare teased PNS fibers, dehydrated nerves were infiltrated with epoxy resin, and the epineurium and perineurium removed. Single fibers were dissected apart with needles, the fibers mounted on glass slides, hardened and a cover slip mounted with epoxy resin. The preparation was then be examined by light microscopy.

Standard Tissue Areas Sampled: These include the plantar, tibial and sciatic nerves, dorsal and ventral lumbar roots, dorsal root ganglia, multiple levels of the spinal cord, medulla oblongata, cerebellum, pons, hypothalamus, optic nerve, lateral geniculate body and superior colliculus. We have observed axon changes in other toxic neuropathies at all of these sites.

Tissue arsenic levels - Samples of urine and hair from intoxicated animals were submitted on two occasions to the Poisonlab (1469 S Holley St., Denver, Colorado) for arsenic analysis.

6. RESULTS: (See appendix)

1) 2 mg/kg - 4 rats intoxicated at this level for 18 months displayed normal growth, and behavior. Microscopic examination after sacrifice revealed no abnormalities in the nervous system. Urine arsenic levels (Table I of Appendix) after 9 months were 2100 mcg/liter (2920 mcg arsenic per gram of creatinine).

2) 5 mg/kg - 4 rats intoxicated at this level for 18 months displayed normal growth and behavior. Microscopic examination after sacrifice revealed no abnormalities in the nervous system. Urine arsenic levels (Table I of Appendix) after 9 months were 2500 mcg/liter (5680 mcg arsenic per gram of creatinine).

3) 10 mg/kg - 4 rats were intoxicated at this level and died after 4 months. Autopsy revealed only bronchial pneumonia and hepatic congestion. A fifth rat was administered 10 mmg/kg and survived for 12 months, with normal growth and no clinical findings. Microscopic examination (illustrated in Fig. I of Appendix) of the nervous system revealed no significant changes. Urine arsenic levels (Table I of Appendix) of this animal after four months revealed 2800mcg/L (9330 mcg of arsenic per gram of arsenic).

4) 15 mg/kg - Two rats were intoxicated at this level and died suddenly after the second injection. Autopsy revealed hemorrhages in the upper gastrointestinal tract and kidneys.

5) Controls - Three control rats received weekly saline injections for 18 months and at time of sacrifice displayed no lesions in the nervous system.

7. SUMMARY AND CONCLUSION:

Intoxication of rats with inorganic arsenic for 18 months has failed to produce nervous system damage despite high levels of total body arsenic. It appears that prolonged intoxication of rats with levels exceeding 5 mg/kg per week is extremely difficult because of high mortality. We succeeded in maintaining one animal for a year at the 10 mg/kg and, despite formidible levels of total body arsenic, no neurotoxicity developed.

There are two possible explantations for the failure of this study to produce neurotoxicity. One is that the rat is immune to the neurotoxic effects of arsenic because of metabolic factors, eg. enzymes necessary for neural maintainence in the rat nervous system are not affected by arsenic. A second explanation is that the life span of the laboratory rat (30 months) is two short to allow adequate prolonged, low-level accumulation of arsenic trioxide sufficient to be neurotoxic. I favor the later explanation, and suggest that future studies of low-level inorganic arsenic intoxication be done in young kittens, and pursued over a 4-5 year period.

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Appendix

Figure 1: Sections of the tibial nerve near calf muscles (a,b) and the gracile fasiculus (c) from rat intoxicated for 12 months with 10 mg/kg/week. These sections display no abnormal features.

Table 1: Report from Poisionlab on urine and hair arsenic analysis of 4 rats from this study. Note highest levels in animal 1 (10 mg) and lesser levels in animals 2 (5 mg), 3 (2 mg) and 4 (control).

POISONLAB #		SPECIMEN #	ARSENIC	CREATININE	ARSENIC/gm CREATININE
66227	1omg	#1 Urine	2800 mcg/L	.3 gms/L	9330 mcg Arsenic / gm Creatinine
66228	5mg	#2 Urine	2500 mcg/L	.44 gms/L	5680 mcg Arsenic / gm Creatinine
66229	2mg	#3 Urine	2100 mcg/L	.72 gms/L	2920 mcg-Arsenic / gm Creatinine
66230	Control	#4 Urine	Less than 20 mcg/L	.4 gms/L	Less than 50 mcg Arsenic / gm Creatinine
66231	1omg	#1 Hair	29 mcg/gm (ppm)		
66232	5mg	#2 Hair	20 mcg/gm (ppm)		
66233	2mg	#3 Hair	12 mcg/gm (ppm)		
66234	Control	#4 Hair	Less than 1 mcg/gm (ppm)		

TABLE I

APPENDIX

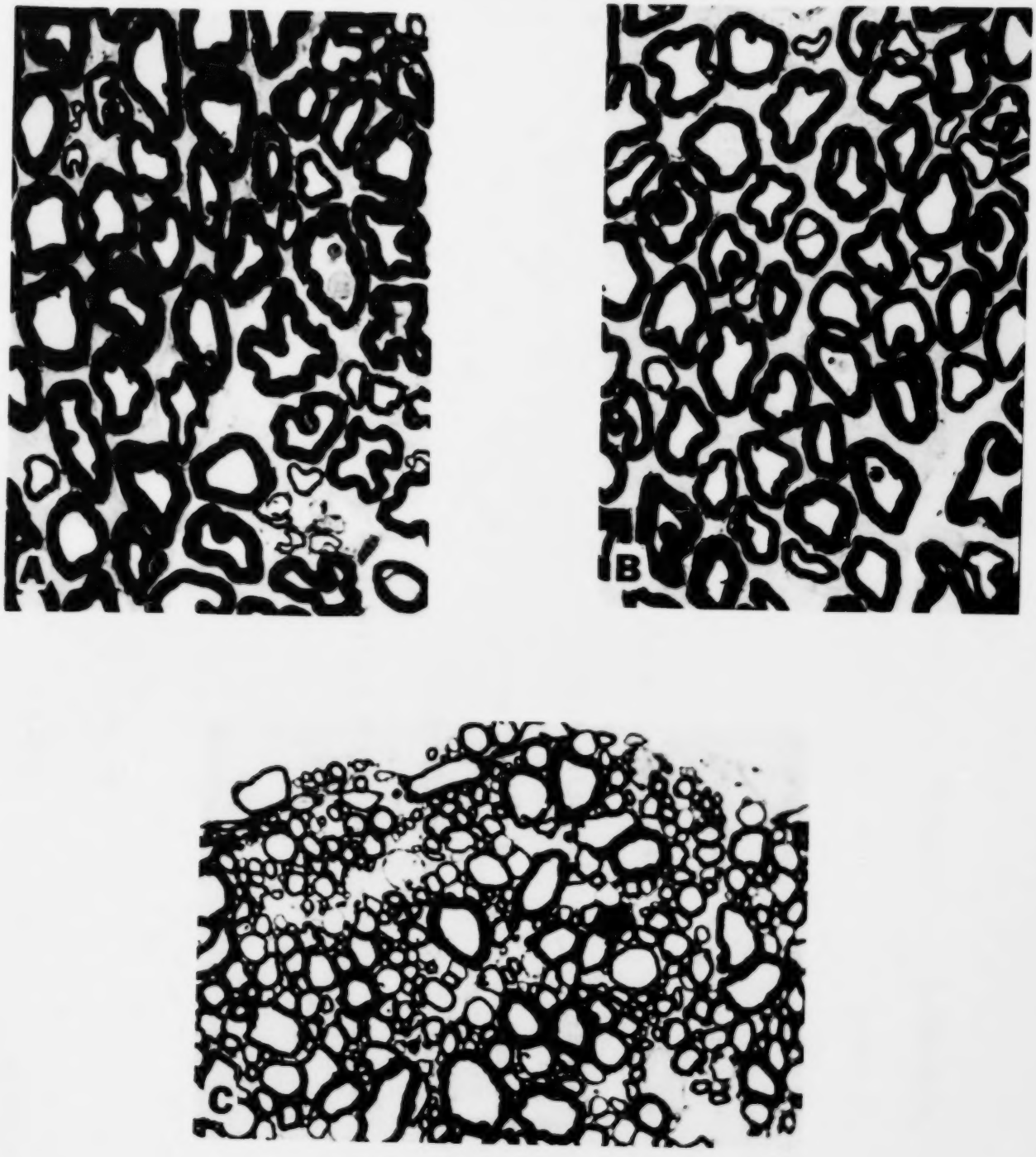

FIG I